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Microscopical studies on exuviae of the jumping spider *Phidippus regius*

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GENERAL NOTE: A preliminary paper on this subject was published in German in ARACHNE 15 (6): 4—11 (2010). Several photographs from that article are used here, with kind permission of the editor of ARACHNE.

1. Introduction

The shed skins (exuviae) of spiders are very practical for morphological studies, because they do not need to be fixed chemically nor dehydrated, but can be examined directly under a microscope. The advantage of using exuviae for light microscopy is that they consist of only exocuticle and are therefore much more transparent than a whole spider. The advantage for scanning electron microscopy is that the dry exuviae can be sputtered directly with gold; any possible shrinkage (due to fixation and dehydration) is thus avoided. Also, there is no need to kill a spider; in fact, since spiders molt several times during their life, it is possible to obtain several exuviae from a single spider. However, it is advisable to use rather fresh exuviae, because if they are stored dry for longer periods of time they may become infected by fungi.

We used mainly the scanning electron microscope (SEM) to study the following body parts of dissected exuviae: a) chelicerae, b) tarsi of legs and palps, c) mouth parts (maxillae, labium) and fore gut, d) corneas of the frontal eyes (anterior medial and anterior lateral eyes).

2. Material and Methods

All the exuviae for this study came from our colleague Judith Kastenmeier (Vienna), who is breeding *Phidippus regius* in terraria at home. Although they were from juvenile spiders, one can already discriminate males from females by the color of their chelicerae: whereas the basal segments are greenish-violet in females, they are strikingly blue and green in males (Figs. 1a, 14).

Since dry exuviae are rather brittle, it is best to dissect them under alcohol. For scanning electron microscopy the isolated parts were then air-dried on filter paper, carefully oriented and glued on aluminum stubs with sticky carbon tape (Fig. 1b) before sputtering them with gold. Specimens were examined in a Zeiss-DSM 950 SEM at 15 or 20 kV, using magnifications from 20 to 10,000 x, and photographed with a Pentax digital camera.

3. Results

a) Chelicerae

The chelicerae of *Phidippus* species have rather stout basal segments and strong cheliceral fangs (Fig.1a). The iridescent green and blue coloration in the male chelicerae is due to a regular fine stacking of the cuticle (Ingram *et al.* 2011). As in many other salticids, only 1—2 cheliceral teeth are present on either

side of the cheliceral furrow, in which the cheliceral fang usually rests (Fig. 2a). The openings of the venom glands are very distinct and lie on the back side of the cheliceral fangs, about 100 µm from the very tip. The posterior margin of the inner side of the cheliceral fang shows a fine serration, that is apparently used for clipping silk threads (Peters, 1982). In fact, we did occasionally find severed strands of silk caught in the little teeth of the cheliceral fangs (Fig. 2c).

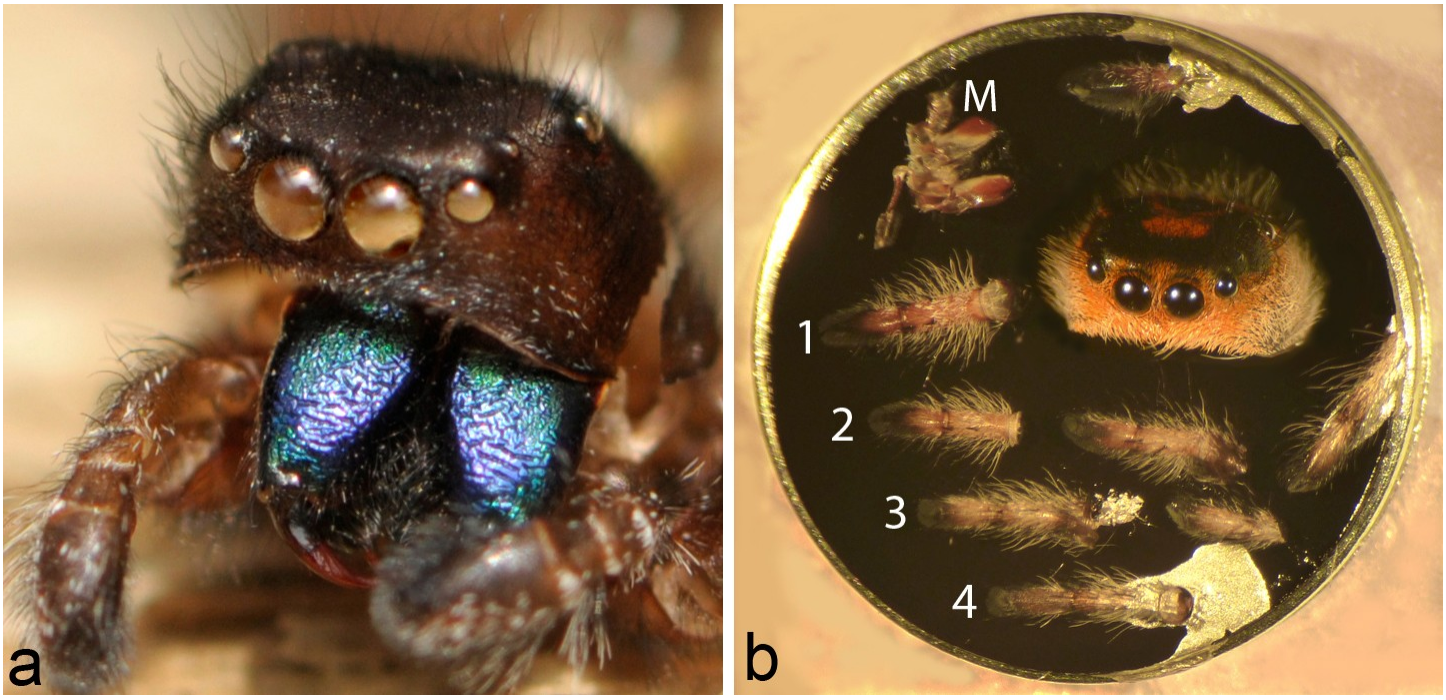

Fig. 1 a) Exuvium of a subadult male *Phidippus regius*. Note the carapace with the shed corneas of the eight eyes and the iridescent bluish-green chelicerae, which are typical for the males. **b)** Various parts of an exuvium of a subadult female *P. regius* mounted on a stub before being sputtered with gold for examination in the scanning electron microscope. The tarsi of legs 1—4 have been mounted ventral side up on sticky carbon tape, together with the carapace and the mouth parts (M).

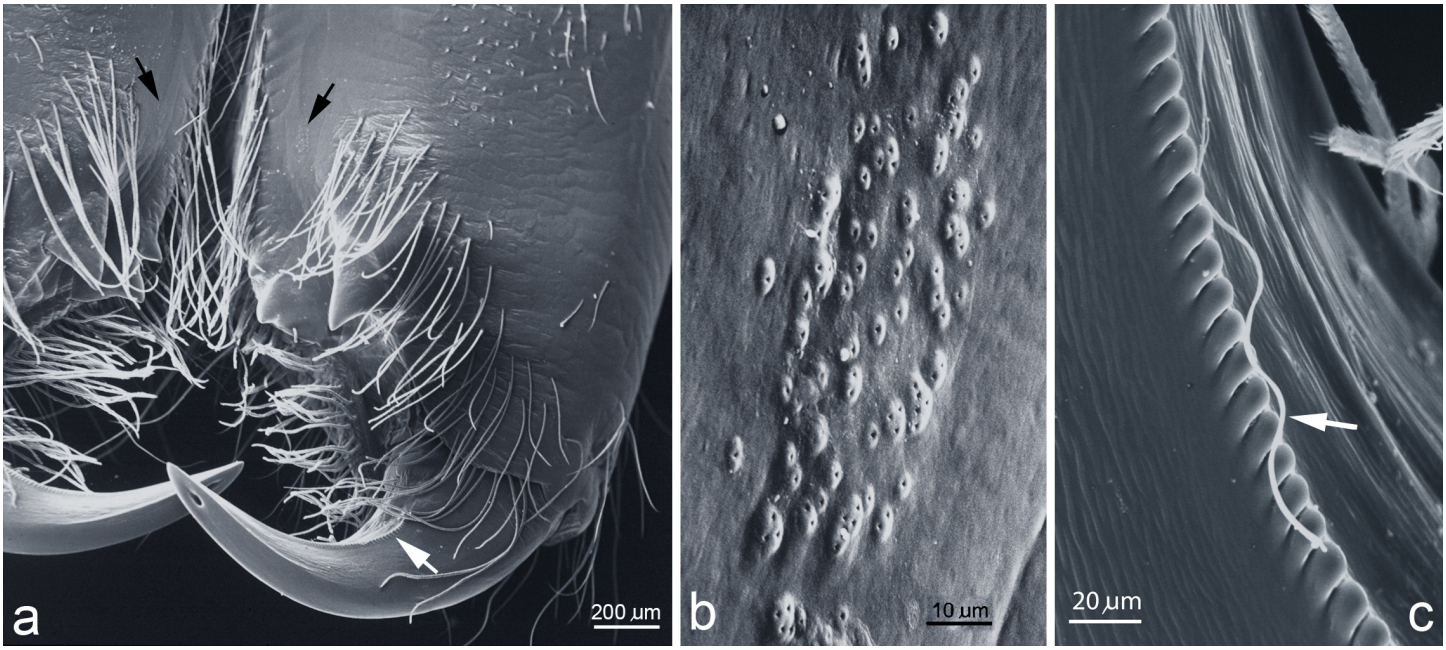

Fig. 2 a) Rear view of the chelicerae. Note the opening of the venom gland near the tip of the cheliceral fang, and the serrated edge (white arrow) on the inside of the cheliceral fang. The black arrows point to an oval field of small pores. **b)** These pore fields contain 70—80 tiny openings of unknown function, **c)** The serrated inner edge of the cheliceral fang is apparently used to clip silk threads (arrow).

A new structure was discovered near the base of the cheliceral furrow, about where the tip of the cheliceral fang comes to rest: an oval field of 70—80 tiny pores (Fig. 2b). So far it is not clear whether these are pore openings of a cheliceral gland or whether they represent sensory structures, *e. g.* gustatory receptors. They can only be seen at high magnification in the SEM, but they are consistently located in the same spot on both chelicerae (Fig. 2a).

By chance we also found some tiny animals wedged between the hairs near the cheliceral furrow (Fig. 3). These were mites of about 150 µm body length. It is an unresolved question, whether they are parasitic (*e. g.* by imbibing hemolymph at the joint membrane of the cheliceral fang) or merely phoretic. Most likely they lived originally on the prey animals (*Drosophila*) which were fed to the spiders. During feeding they may have simply switched their host, but it is not clear whether they would then permanently live on the spider. At any rate, after ecdysis of the spider the mites were stuck on the shed skin.

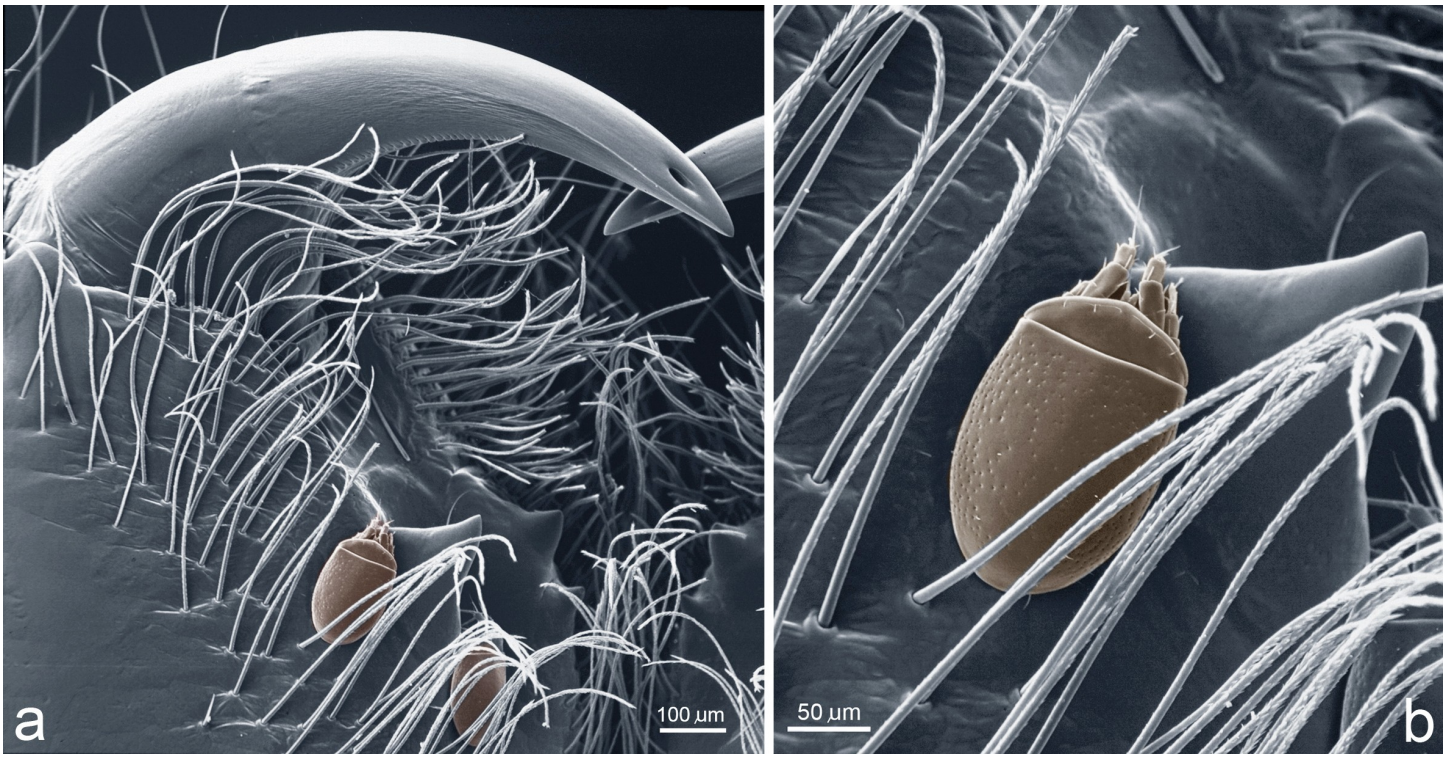

Fig. 3 a, b In some exuviae small mites (marked in brown) were found on the chelicerae. It is not clear whether these mites are parasitic or merely phoretic. Most likely they lived originally on the prey animals (*Drosophila*) that were offered as food.

b) Tarsi of legs and palps

We were mostly interested in the claws, the claw tufts and the adhesive hairs on the ventral side of the tarsus, but had only a brief look at the various sensory hairs.

Claws: The two tarsal claws are prominent yet difficult to see, because they are largely hidden behind the extensive claw tufts. Only if a tarsus is split in half longitudinally, is it possible to have an unobstructed view of the two claws arising from the pretarsal plate (Fig. 4). This procedure was first used by Hill (1977) to demonstrate the movable pretarsus, including the attached cuticular tendons: the upper tendon belongs to the *musculus levator pretarsi* which can lift the entire pretarsus, whereas the lower tendon belonging to the *musculus depressor pretarsi* depresses the pretarsus.

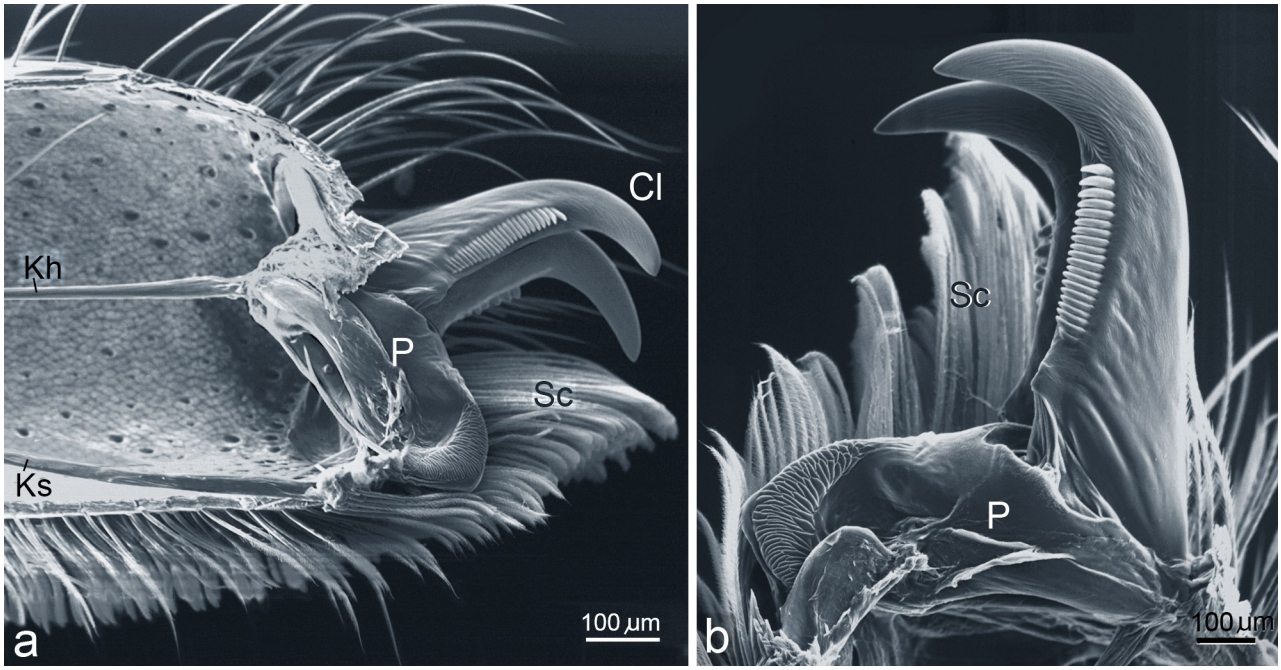

Fig. 4 a) A longitudinally split tarsus from an exuvium shows two combed claws (cl) arising from a pretarsal plate (P). The claws can be raised by the action of a cuticular tendon (Kh) that inserts on the top of the pretarsus, and lowered by the action of a tendon (Ks) that is attached tot he bottom oft he pretarsus. Sc = scopula hairs of one claw tuft. **b)** The two claws have a different number of teeth: the anterior claw has many fine teeth, whereas the posterior claw has only a few teeth.

The fact that these tendons are shed during a molt is a bit surprising, even to biologists. In the living state the tendons are covered by a thin epithelium that secretes the cuticle toward the inside, forming a long tube. During ecdysis these epithelial cells separate from the cuticular tube in the same way as the hypodermis cells separate from the external cuticle. While a new thin cuticle layer is produced into the gap, the old cuticular tendon is pulled out together with the claws (and the entire exoskeleton) during the actual molt. The same procedure happens with other internal cuticular structures such as the fore gut (see below) or the cuticular lamellae of the book lungs.

The two claws both have a comb structure on the inner margin; however, the anterior claw exhibits many more teeth (over 20) than the posterior claw (5—8). Although it is known that the claws are used to handle silk threads, it is still not quite clear what functional significance can be assigned to the fine teeth on the anterior claws as compared to the coarse teeth on the posterior claw (Hill, 2006). It is interesting, however, that the interstices of the fine teeth would perfectly match the diameter of silk threads (1—4 µm; see Figs. 11 and 12 in Hill, 2006). A similar matching of thread diameter and serrated bristles has been noted in araneids, pholcids and theridiids (Figs. 2.12 and 4.3 in Foelix, 2011). As a working hypothesis Hill (2006) has suggested that the coarse teeth of the posterior claws would be used for handling and releasing silk threads quickly, while the fine teeth of the anterior claws might provide a firm and more secure grip. It should be pointed out though that the combed main claws in araneids are not involved in grasping silk threads: an ablation of the combed main claws on all legs did not affect the spider´s ability to climb on silk threads. However, these web spiders are special in having a distinct third claw (middle hook) which is apparently specialized for grasping and climbing silk threads (Foelix, 1970a).

Claw tufts and scopulae: The typical claw tufts are paired foot pads, usually consisting of several hundred tightly packed adhesive hairs. They are located directly under the claws and also arise from the pretarsal plate (Hill, 1977, 2006). Often similar adhesive hairs are also present on the ventral side of the tarsus, but not as densely packed as in the claw tufts. They form so-called scopulae, usually as two strips on the ventro-lateral side of the front legs (Fig. 5). These scopulae are lacking on the hind legs (3 and 4), where only well-developed claw tufts are found. Actually the claw tufts on the hind legs display many more

adhesive hairs than on the front legs. We counted about 220 adhesive hairs in one foot pad of leg 1 and 380 on leg 4 (Fig. 6); Hill (2006) reported about 180 adhesive hairs in one foot pad of leg 1, and about 300 on leg 4 for an adult male *Phidippus audax*.

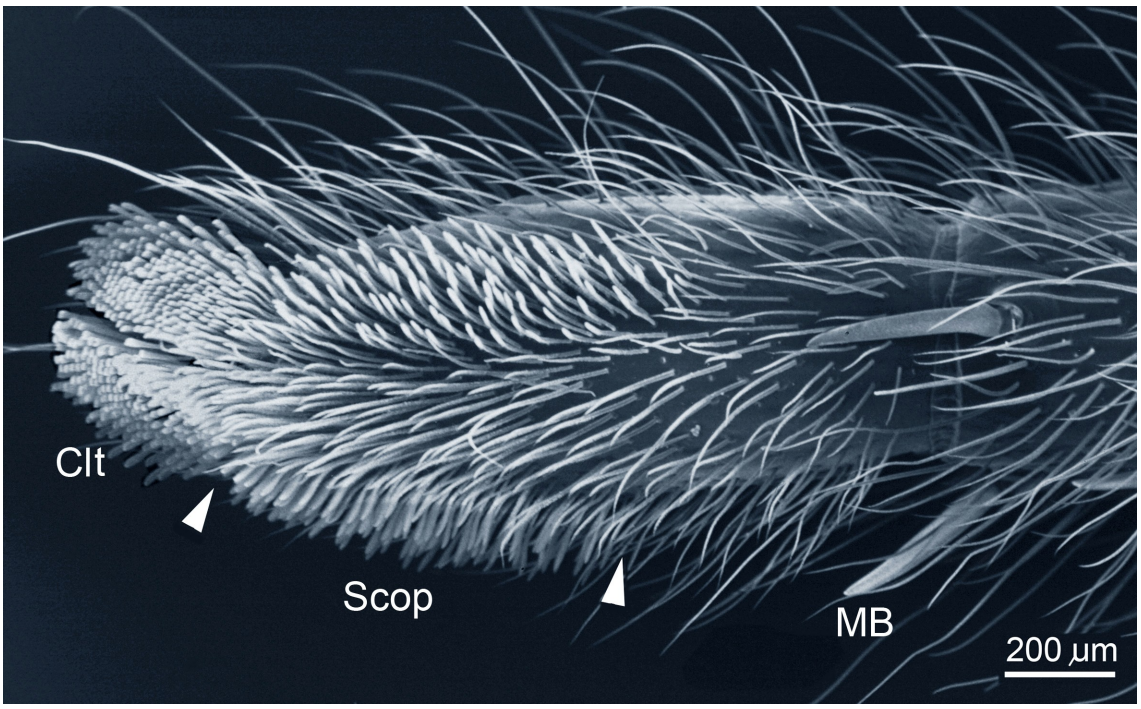

Fig. 5 Tarsus of leg 2 in ventral view. Two claw tufts (clt) are symmetrically arranged at the tarsal end. Further adhesive hairs (Scop) extend on the ventral surface of the tarsus. Two stout metatarsal spines (MB) form a catching basket near the tarsus-metatarsus joint.

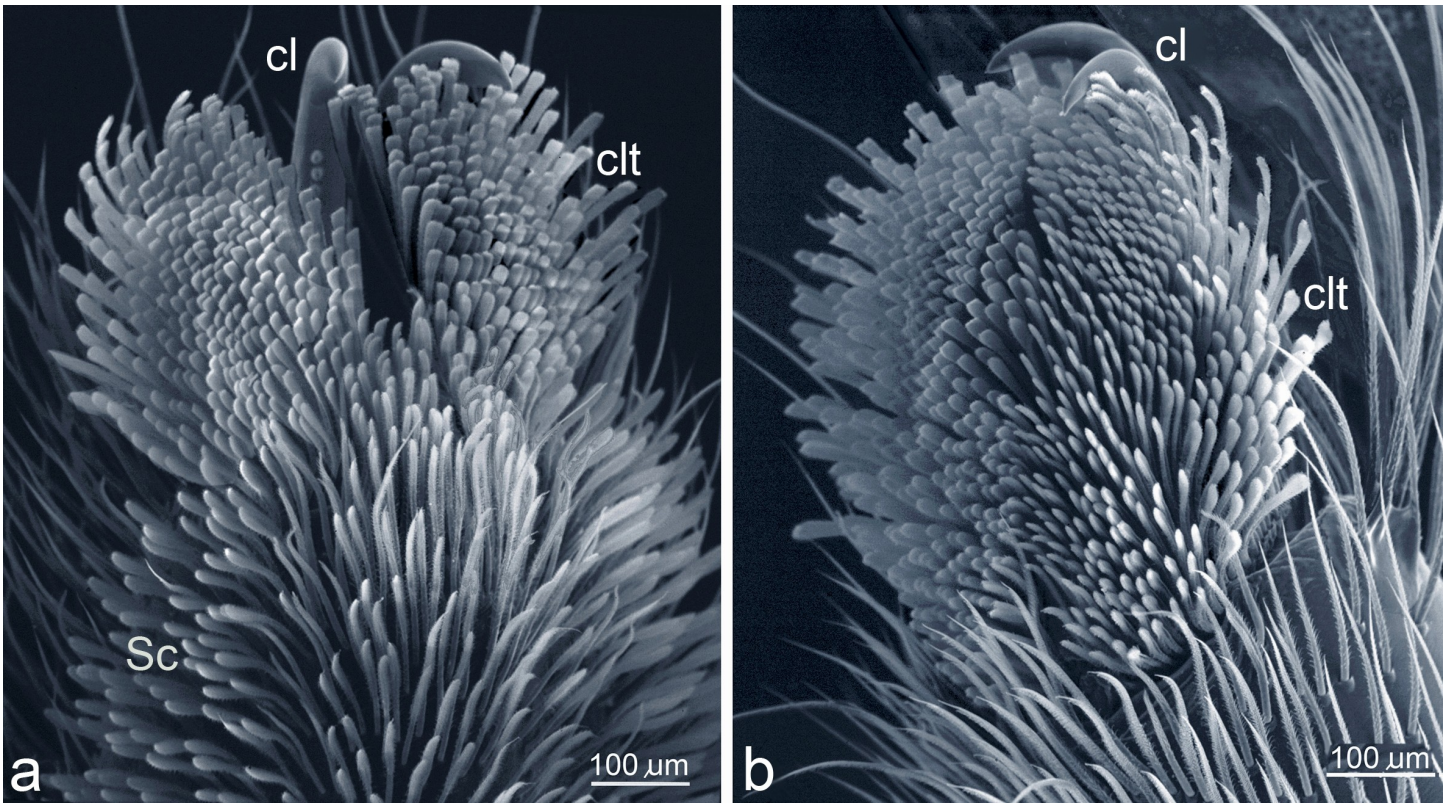

Fig. 6 Comparison of tarsal tips in front and hind legs, ventral view. **a)** Tarsus 1 has two distinct claw tufts (clt) beneath the claws (cl), and additional ahesive hairs (sc) on most of the ventral surface. **b)** Tarsus 4 exhibits only claw tufts but lacks scopulae. Note that the claw tufts are bigger than on tarsus 1.

It is well known that the claw tufts are used for walking sure-footedly — even upside-down — on smooth surfaces (Homann, 1957; Hill, 2006; Kesel *et al.*, 2003). The scopulae on the ventral tarsi are probably more involved in prey capture, securing a firm grip on the victim (Rovner, 1978; Foelix *et al.*, 1984; Niederegger & Gorb, 2006). In this respect it seems logical that salticids have scopulae only on legs 1 and 2, since they seize their prey only with their front legs.

In one exceptional case, when we compared the left and right leg 1 of the same spider, we noticed the regular set of 2 claw tufts plus scopulae on the left tarsus, but only small claw tufts on the right tarsus (Fig. 7). Our interpretation is that the right first leg was probably a regenerate, in which the scopulae had not been developed. It is known from regeneration experiments that certain sense organs (*e. g.* the tarsal organs) are still lacking in the first regenerate and that the number of sensory hairs is reduced (Blumenthal, 1935).

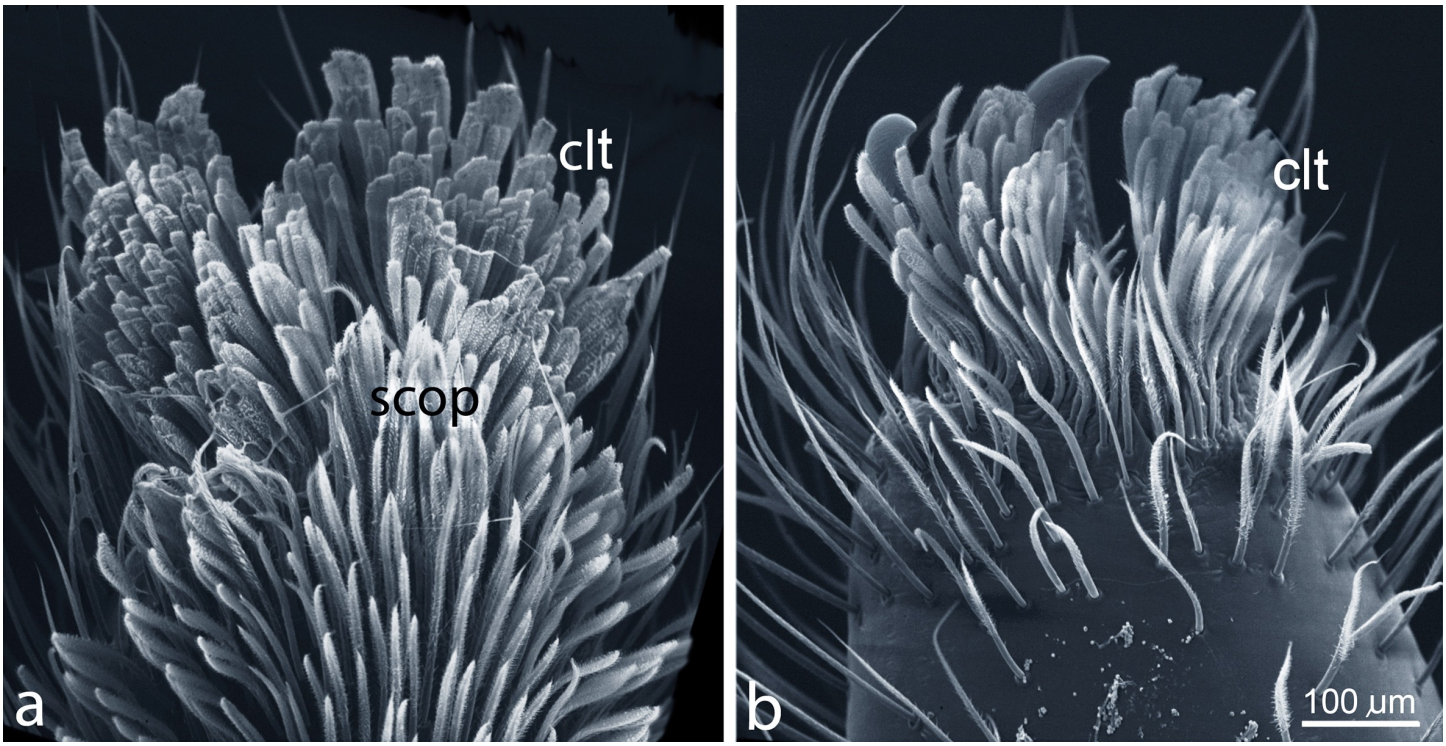

Fig. 7 Ventral view of the first tarsi in the same spider. The left tarsus **(a)** shows claw tufts and scopulate hairs, but the right tarsus **(b)** only claw tufts. Most likely the right tarsus is a regenerate in which the scopula has not yet been formed.

Metatarsal spines (macrosetae): Two strong bristles are located ventrally on the distal metatarsus, bridging the joint with the tarsus. These spines play apparently an important role during prey capture, acting as a kind of catching basket (Hill, 2006). Each spine is over half a millimeter long and measures between 60 and 70 µm in diameter (Figs. 5, 8). At higher magnification under the SEM distinct ridges become visible on the inner surface of the bristle shaft; in contrast, the outer surface is covered with rows of small denticles (Fig. 8b). It seems reasonable that the highly textured surface of these spines provides enhanced friction, which would be of advantage when grabbing a prey animal.

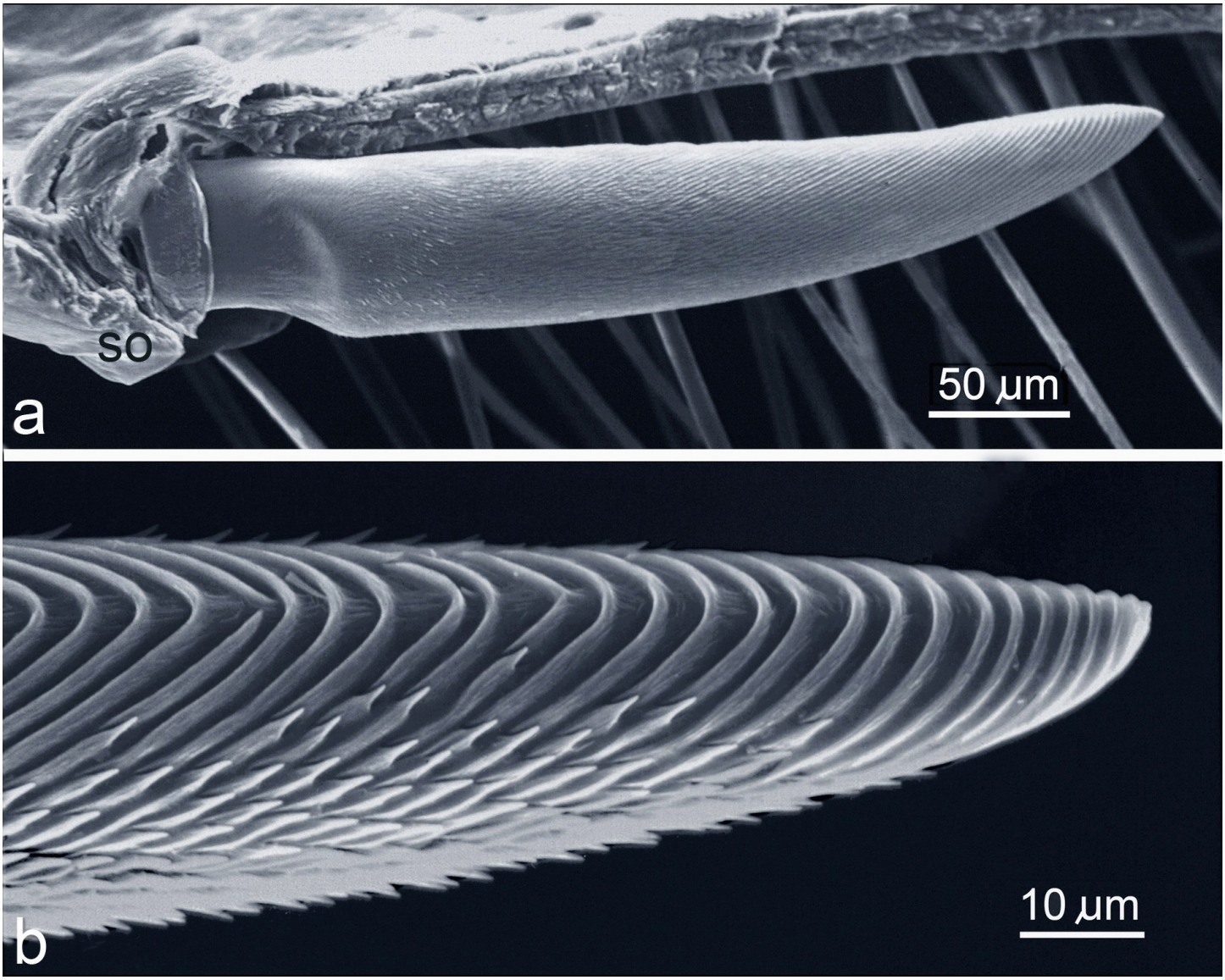

Fig. 8 a) Strong metatarsal spines are more than 500 µm long and 60-70 µm in diameter. The hair shaft is movably articulated in a cuticular socket (so). **b)** The hair shaft has a fingerprint-like pattern of ridges on the inner side and many fine teeth on the outer side.

Chemoreceptors: The main chemoreceptors are specialised hairs with an open tip (Foelix, 1970b, Foelix & Chu-Wang, 1973). The actual pore at the blunt tip is difficult to demonstrate (less than 0.5 µm diameter, often clogged), but there are other diagnostic features that are easier to see: the hair shaft is bent into a S-shape and its distal part shows a spiral structure, often with little cuticular extensions (Fig. 9a, b). Therefore these hairs were referred to as "whorled setae" or "spondylae" by Hill (1977, 2006). They are concentrated at the tips of the extremities (palps, legs, mouth parts and even on the spinnerets), usually more on the ventral side. In salticids they surround the claw tufts and the scopulae, so that contact with the substrate is ensured. Based on behavioral observations Bristowe (1941) had coined the term "taste-by-touch" sense long ago, without knowing which receptors would actually be responsible for this contact chemoreception.

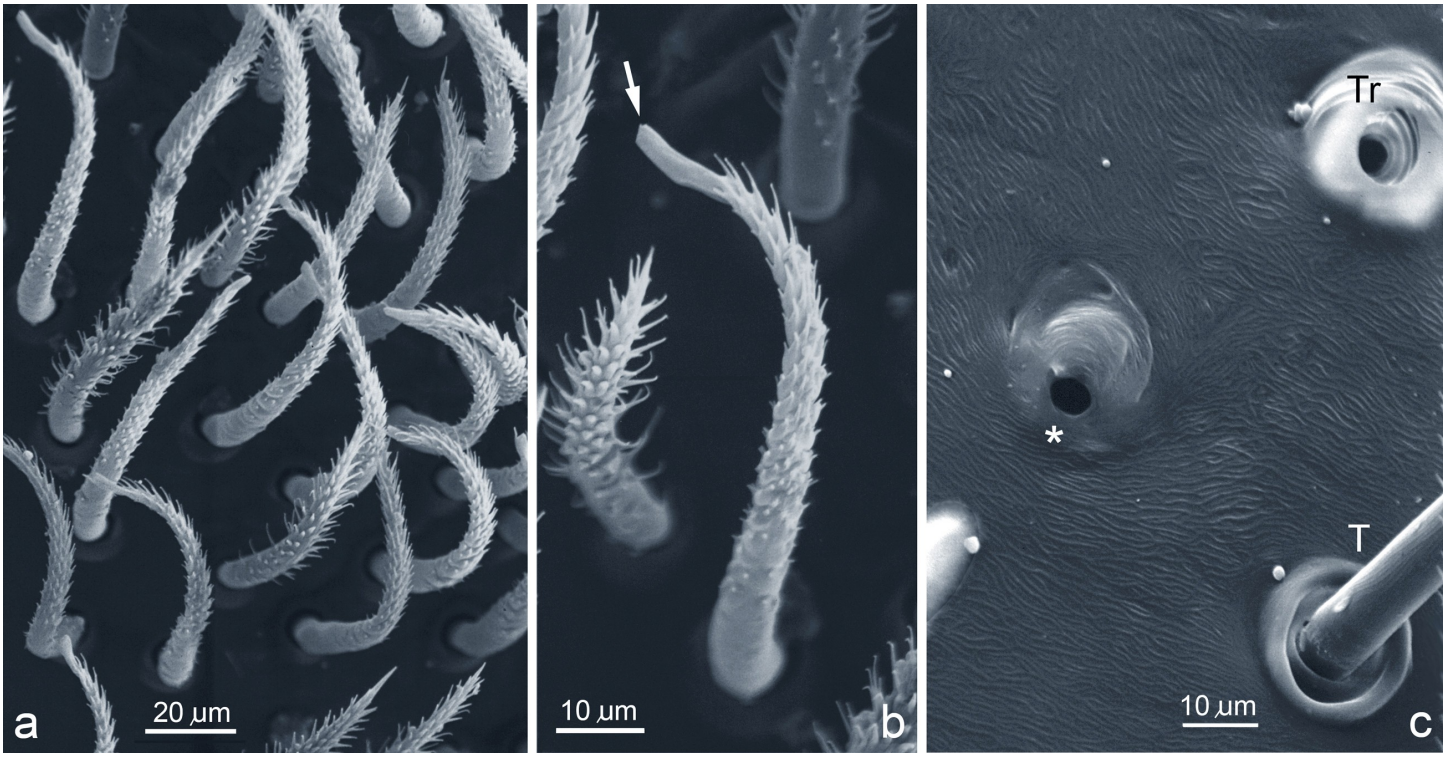

Fig. 9 Chemoreceptors. **a)** The palpal tips are densely covered with chemosensitive hairs. **b)** Typical for chemosensitive sensilla is a bent hair shaft which is smooth at the base but bears spiny extensions more distally. The hair tip (arrow) is blunt and exhibits a small pore that connects the outside with dendritic nerve fibers inside the hair shaft. **c)** The tarsal organ is located dorsally near the tarsal tip; superficially it resembles an empty hair base — compare with the bases of a trichobothrium (Tr) and a tactile hair (T).

On the palps of *Phidippus* these chemosensitive hairs occupy mostly the dorso-lateral sides of the tarsus, where they most easily come into contact with the environment (Figs. 9a, 14c). The inner (ventral) surface of the palps bears some special cleaning hairs which will be discussed below.

Another chemoreceptor, or better said, hygroreceptor, is the *tarsal organ* that occurs on all legs and palps on the dorsal side of the tarsus (Blumenthal, 1935; Foelix, 1970b; Ehn & Tichy, 1994). In *Phidippus* the tarsal organ is difficult to find because it is very small and is easily mistaken for an empty hair socket (Fig. 9c). Also, it is located very distally on the tarsus, right behind the claws, whereas in most araneomorph spiders it lies more proximally. The sensitive nerve endings lie inside the little pit and therefore cannot be seen from the outside with the SEM.

c) Mouthparts

The mouthparts consist of the two enlarged palp coxae, the maxillae (endites), and in between the small labium (Fig. 10a). The lateral rim of each maxilla is differentiated into a saw-like structure, the serrula. It is assumed that this sharp edge is used to cut into the prey to facilitate the external digestion; however, to our knowledge actual observations or detailed descriptions of this action are lacking.

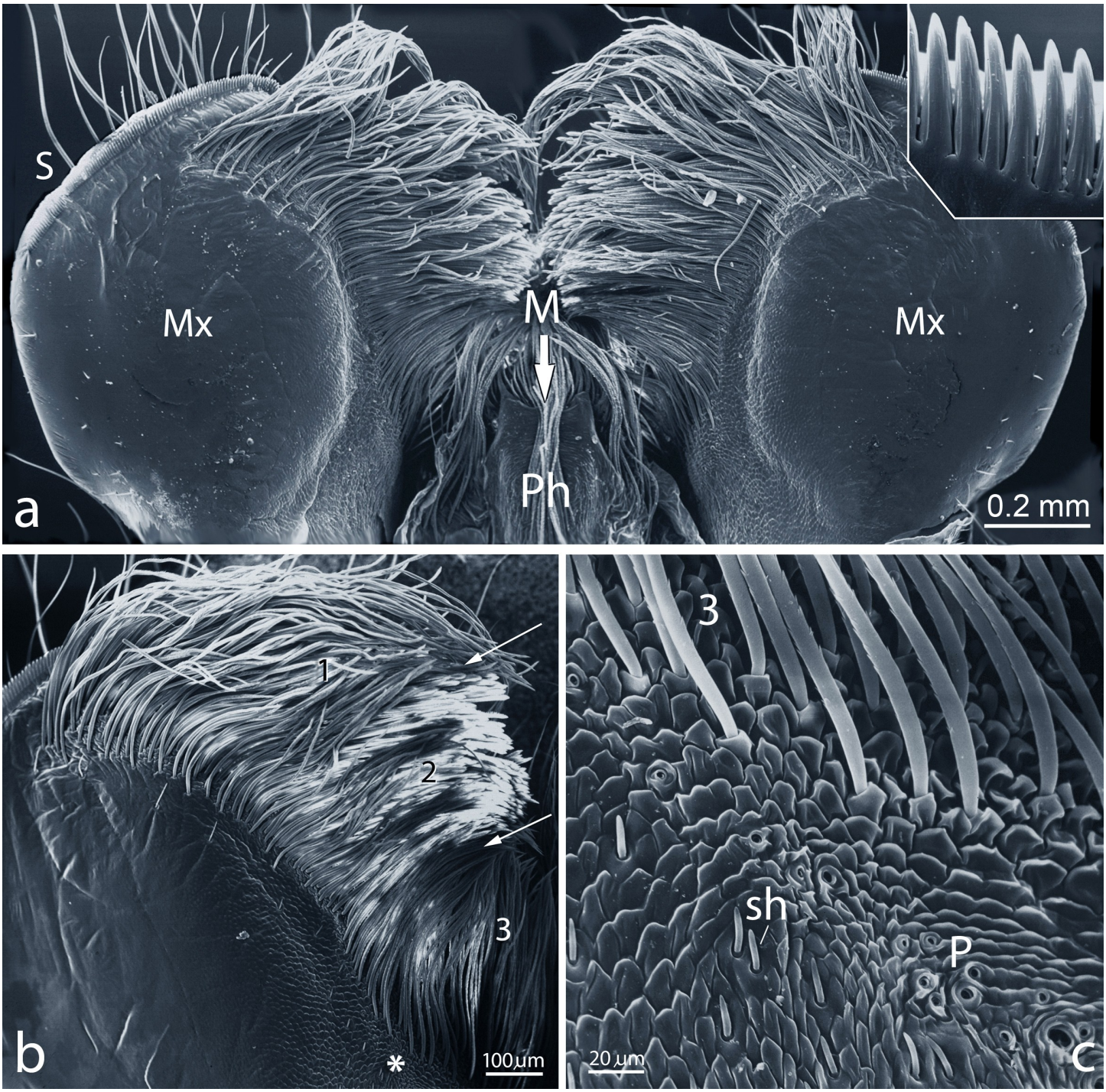

Fig. 10 a) Mouth parts seen from the inside. The anterior rim of the maxilla (Mx) is differentiated into a saw-like serrula (S, see enlarged in the Inset); the medial side is covered by filtering hairs. The small mouth opening (M) lies in the center and leads into the flat pharynx (Ph). **b)** Left maxilla, inside. Numerous filtering hairs on the medial side belong to three different types: (1) long wavy hairs, (2) shorter, club-shaped hairs, and (3) thin, spiky hairs. Laterally to the type 3 hairs lies a field of small pores (*). **c)** These pores (P) are shown here in higher magnification; they represent the openings of a salivary gland that lies inside the maxilla. Tiny sensory hairs (sh) are distributed over the adjacent cuticle.

We focused our attention on the more interesting internal side of the mouth parts. A fringe of many dense hairs occupies the medial side of both maxillae. They are generally considered as filtering hairs when the predigested, liquified prey is sucked in. It was known that these filtering hairs have a brush-like appearance, which would help in retaining larger food particles. A closer inspection of these filtering hairs with the light microscope actually revealed 3 different hair types (Fig. 11): a) a group of long sinuous hairs lying distally, b) a group of shorter, straight but club-shaped hairs lying in the middle, and c) a group of thin, spiny hairs lying proximally and also extending onto the labium (Fig. 10b). In close proximity to that third group of hairs is a small field of about 14 pores (Fig. 10c); these represent most likely the openings of a salivary gland which is located inside the maxilla (Foelix 1970b). The 3 types of filtering hairs were examined in more detail with the SEM (Fig. 12) and it seems that the type 2 with its broom-like distal end may play the main role in the filtering process.

Fig. 11 The 3 types of filtering hairs as seen in the light microscope. **a)** type 1, curved hairs ending in long barbs, **b)** type 2, straight hairs with barbed, club-shaped endings, **c)** type 3 , thin, spiky hairs.

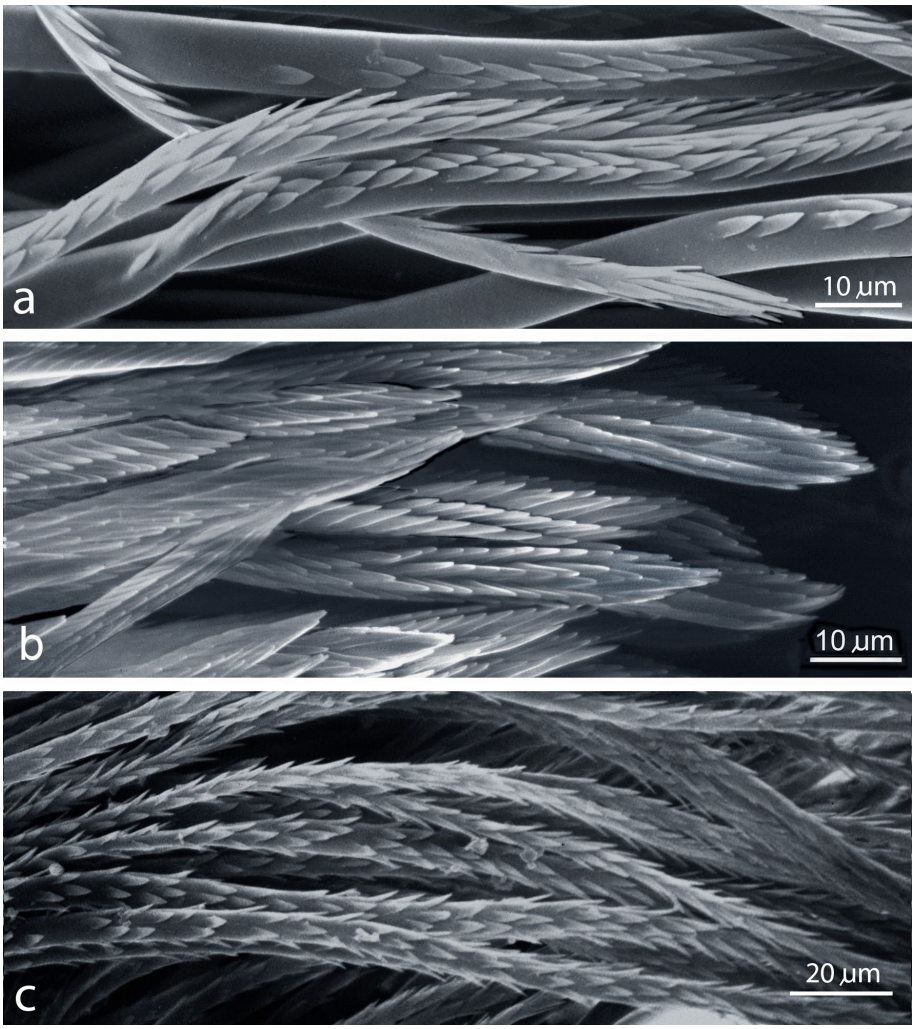

Fig. 12 The same 3 types of filtering hairs seen in the SEM. **a)** type 1, with few barbs on only one side of the hair shaft, **b)** type 2 hairs resembling tiny tooth brushes, **c)** type 3 hairs with many fine barbs along the entire hair shaft.

Fore gut: Since we were examining exuviae, all the cuticle-lined parts of the fore gut were also preserved, *i. e.* the flattened pharynx, the narrow U-shaped tube of the esophagus, and the collapsed walls of the sucking stomach (Fig. 13). With the aid of the SEM we could detect details like the filtering lamellae inside the pharynx or the dimpled surface of the sucking stomach, where the dilation muscles had inserted. An overview of the mouth parts and the attached fore gut also demonstrates nicely where the actual mouth opening of the spider is situated.

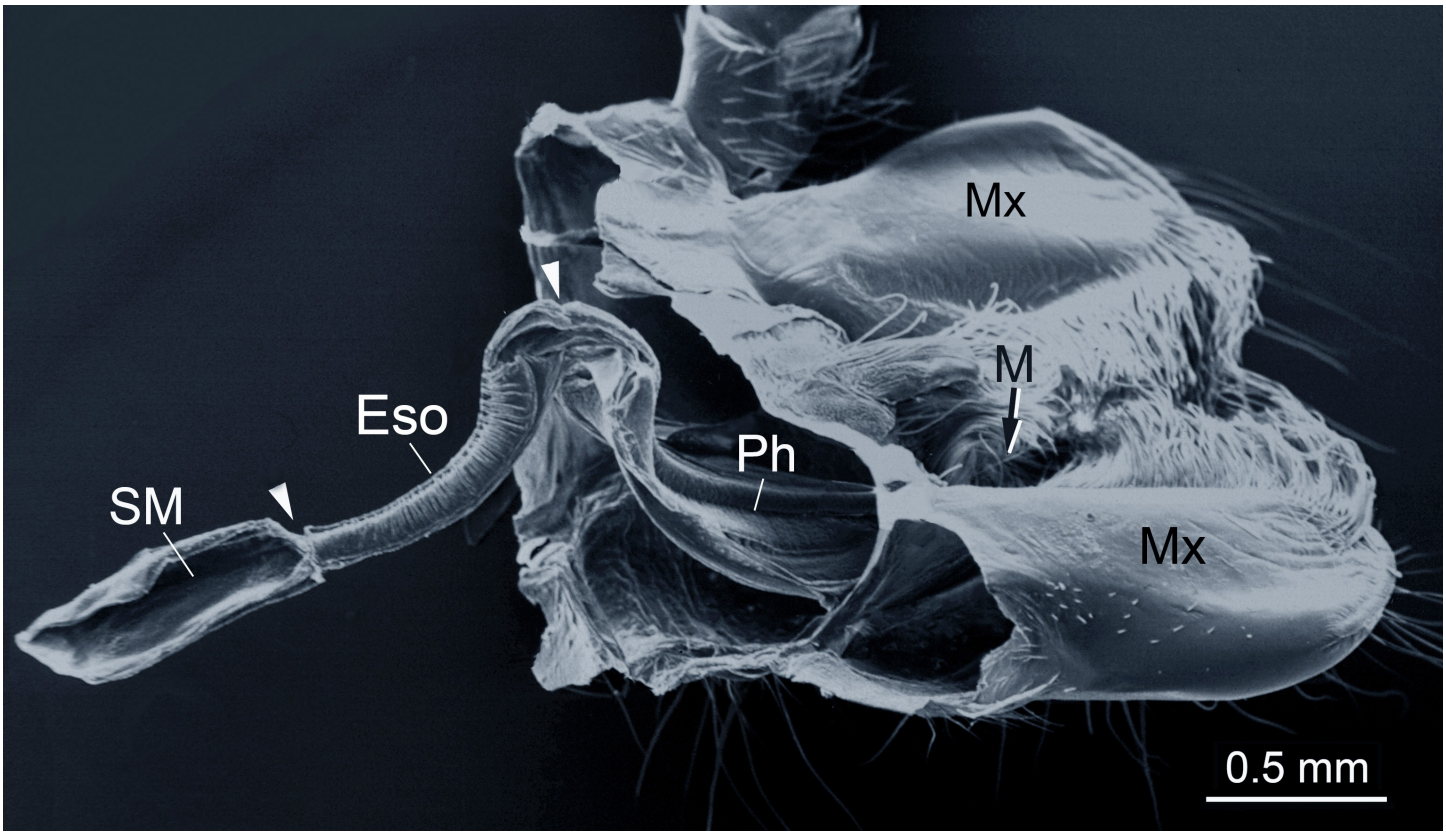

Fig. 13 A lateral view of the mouth parts of an exuvium shows that the internal parts of the fore gut (pharynx Ph, esophagus Eso, sucking stomach SM), which are lined with a thin cuticle, are shed during a molt.

d) Eye lenses and cleaning devices

When watching a live jumping spider (Fig. 14a) one can easily notice how they often wipe their front eyes with the inside of their pedipalps. This is particularly conspicuous when a male courts a female. We wondered whether the palps might be equipped with some special cleaning hairs and examined the medial side of the pedipalps under the microscope. Already at low magnification a group of dark hairs becomes visible on the inside, which is in stark contrast to the long white hairs covering the rest of the palp (Fig. 14b). At higher magnification these hairs appear like thin brushes, somewhat similar to the type 3 filtering hairs on the maxillae (Fig. 14c).

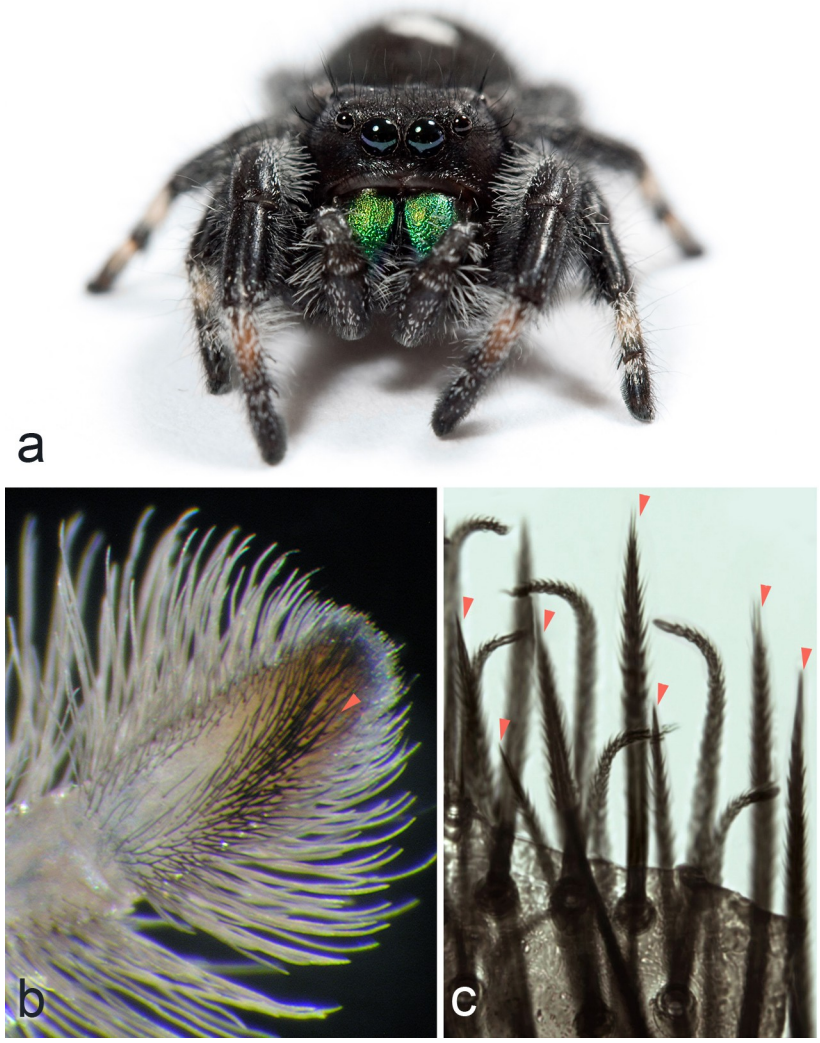

Fig. 14 a) Frontal view of a male of *Phidippus regius*. The iridescent green chelicerae are partly covered by the palps. Photo: B. Rast. **b)** Tip of palp, ventral view. Long white hairs cover the dorsal and ventral sides; the dark hairs (arrowhead) are used for wiping the lenses of the front eyes. **c)** Light microscopical view of the palpal cleaning hairs (arrowheads); the bent hairs represent contact chemoreceptors.

The eye lens, or at least the outermost layer, the cornea, is shed during a molt and can thus be easily examined in an exuvium (Fig. 15a). Although the corneal surface seems completely smooth when viewed under low to medium magnifications, a fine pattern of ridges appears at magnifications of 5,000—10,000 x. These ridges are only about 200 nm wide and run parallel to each other in a vertical direction (Fig. 15b). This is true for all eyes, but the pattern may be be more or less distinct in different eyes. We found these corneal ridges also in other salticids (*e. g.* in *Portia*) and also in lycosids (*Lycosa*). It is interesting that a similar corneal differentiation is known from certain compound eyes in insects, *e. g.* in butterflies. However, instead of ridges they exhibit little nipples of approximately the same diameter (Fig. 16). It is believed that this corneal surface grating functions as an anti-glare device (M. Land, W. Ribi, pers. comm.) and it is tempting to speculate that the ridges on the spider corneae may serve the same purpose.

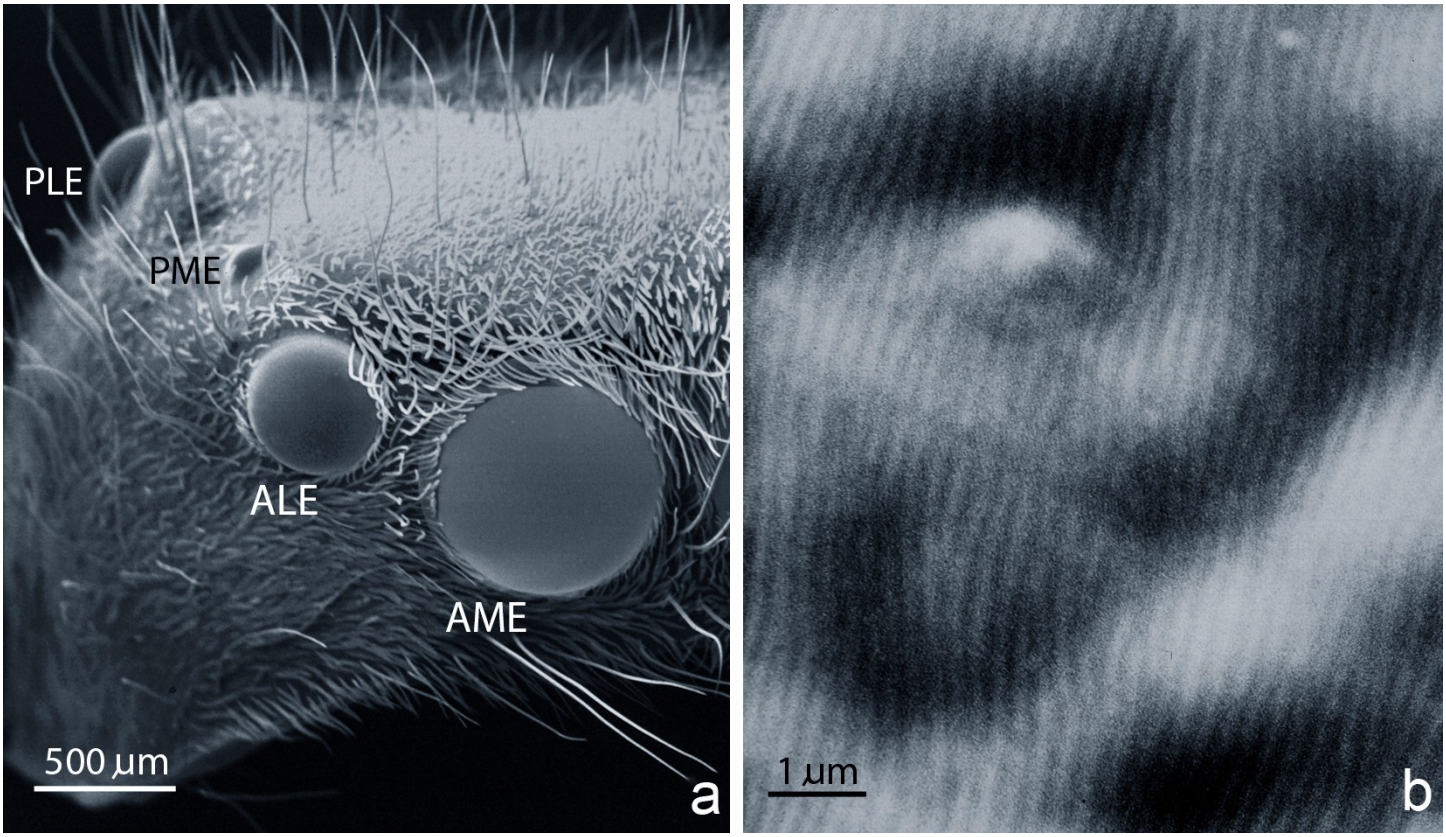

Fig. 15 a) The four different eyes on the right half of a carapace of *P. regius*. Anterior Medial Eyes (AME), Anterior Lateral Eyes (ALE), Posterior Medial Eyes (PME), and Posterior Lateral Eyes (PLE). The lenses of the front eyes (ALE, AME) are cleaned with the inside of the palps (see Fig. 14). The surface of the eye lenses appear completely smooth at low magnification. **b)** A system of small ridges running vertically over the surface of each lens becomes visible at high magnifications.

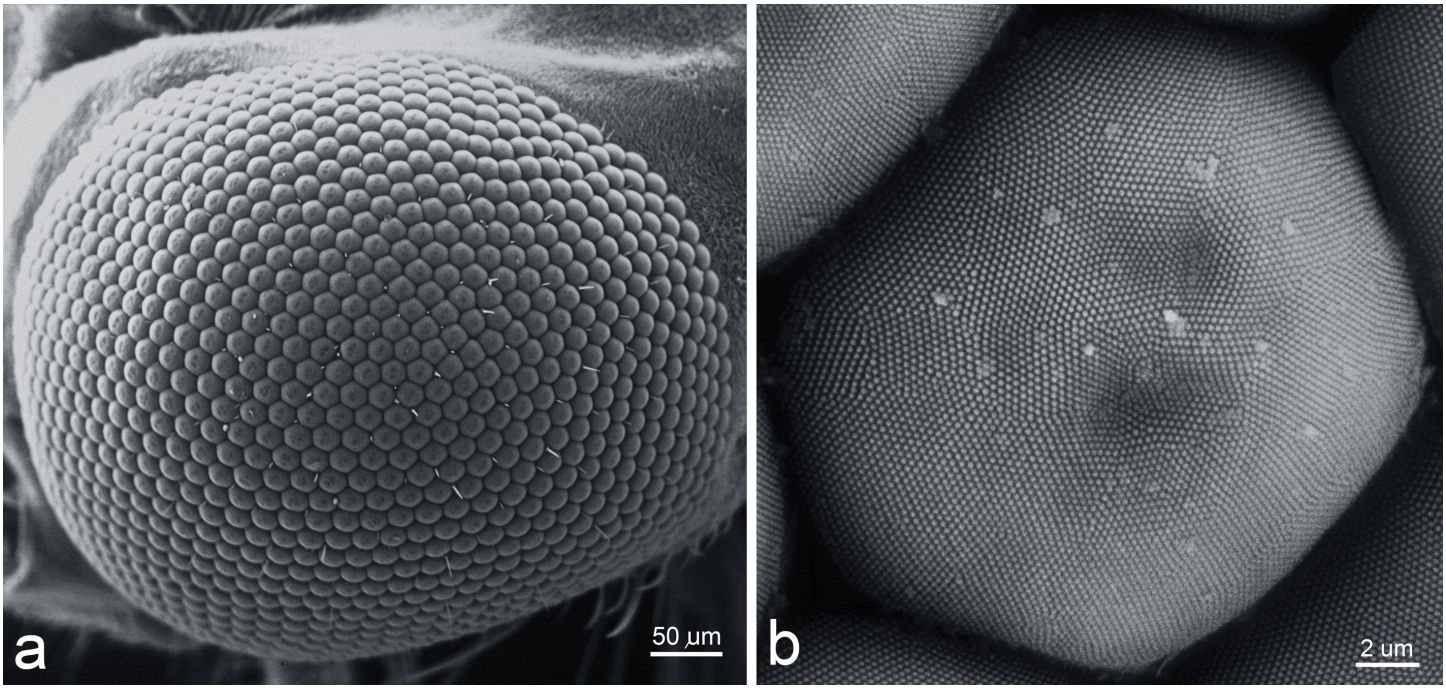

Fig. 16 a) Compound eye of a domestic moth, consisting of about 800 ommatidia. **b)** High magnification of the cornea of a single ommatidium showing thousands of regularly arranged nipples — in the same size range as the ridges in spider eyes.

4. Discussion and Summary

This morphological survey of salticid exuviae has revealed some little known or completely unknown structures, which certainly deserve further studies, especially on the functional level. On the chelicerae we discovered a field of small pores next to the end of the cheliceral furrow that had not been described so far. These pores are also present on the chelicerae of all the other araneomorph spiders that we have examined (we have not checked for their presence in orthognath spiders yet). Since those pores are in close proximity to the tip of the cheliceral fang, it could well be that they are sensory pores (gustatory?), or glandular pores, where some secretion is given off. One possibility is that the secretion may be involved in some kind of "chemical" cleaning, *e. g.* when the tips of the legs are chewed with the chelicerae (Hill, 2010, pers. comm.). Another point with respect to the chelicerae is the serrated inner edge of the cheliceral fang; the supposed function of clipping silk threads (Peters, 1982) needs to be confirmed.

The mouth parts also need to be studied in more detail. For instance, the seemingly obvious function of the serrula as a cutting tool needs to be ascertained by direct observation. And, do spiders which only suck their prey (e.g. thomisids or uloborids) have serrulas nevertheless?

To our knowledge the presence of different kinds of filtering hairs on the maxillae has not been reported before. Are these 3 different hair types specialized for filtering different sizes of food particles? An inspection of these filtering hairs during various phases of feeding might provide an answer. The glandular openings next to the filter hairs (type 3) also deserve further attention: Is it simply some kind of saliva that is secreted, or are digestive enzymes involved (perhaps for cleaning the filtering hairs after feeding)?

Finally, the corneal surface of spider eyes in general should be surveyed for the presence of those ridges – are they found in all spiders or only in diurnal species (which one would assume if they function as an anti-glare device)? In insects a comparable surface structure is found on the corneas of butterfly compound eyes, but not in bees or ants (personal observations), so the situation seems more complex.

In short, the morphological examination of spider exuviae seems well worth the effort, since even completely new structures may be discovered. However, physiological studies are definitely needed to elucidate the possible function of these structures.

5. Acknowledgements

We would like to thank several colleagues for their support: Judith Kastenmeier for supplying the exuviae of *Phidippus regius*, Bastian Rast for his excellent photograph of a male *P. regius*, Michael Land and Willi Ribi for their ideas on the structure of corneas, and David Hill for many discussions of jumping spider biology.

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